IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

 Application of:
 Rice et al.
 Group No.:
 1648

 Serial No.:
 09/576,989
 Atty. Docket No.:
 56029-4356

 Filed:
 05/23/2000
 Examiner:
 Wortman, Donus C.

DECLARATION OF DR. KERIL J. BLIGHT UNDER 37 C.F.R. §1.131

- I, Dr. Keril J. Blight, declare and state as follows:
- All of the statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true.
- I am a co-inventor of U.S. Patent Application No. 09/576,989 for HCV Variants, filed May 23, 2000 (Patent Application).
- 3. I, along with co-inventor Dr. Charles M. Rice (hereinafter, "we"), conceived of, and reduced to practice the inventions claimed in the Patent Application before March 31, 2000.

Ú,

4. Specifically, before March 31, 2000 we identified the adaptive mutations that are described in the Patent Application. Those adaptive mutations are referenced in the attached laboratory notebook pages and computer printouts attached as Exhibit A. The terminology used to describe the cell colonies harboring HCV comprising those mutations in Exhibit A (see A25) corresponds to the terminology used in the Patent Application (see Figure 7) as follows:

BBI	HCVrep1b/Ava.1
BBII	HCVrep1b/Ava.5
BBIII	HCVrep1b/Huh.2
BBIV	HCVrep1b/Ava.7
BBV	HCVrep1b/Ava.2
BBVI	HCVrep1b/Clone A
BBVII	HCVrep1b/Clone R

5. Because the cell colonies were G418 resistant, we expected that the resistance was conferred by HCV replicons comprising adaptive mutations, harbored by those colonies. We tested this theory by sequencing the replicons, which were amplified from cDNA reverse transcribed from RNA isolated from each of the independent G418 resistant cell clones, before March 31, 2000. That data is presented at A3-A19. We then engineered each mutation back into the HCVrep1bBartMan/Avail backbone, as described in the Example in the Patent Application. We then transcribed RNA from each reconstructed replicon and electroporated it into naïve Huh7 cells, and compared the number of G418 resistant colonies compared to that obtained for the HCVrep1bBartMan/Avail replicon containing wild type NS5A (see A1, for example, where it was determined that the mutation identified in clone BBI was capable of increasing the frequency of G418 resistant colonies). Based on that result, we reasonably expected that the other mutations identified would similarly confer increased frequency of G418 resistant colonies, due to increased transfection efficiency of the mutant HCV.

7. I understand that willful false statements and the like arc punishable by fine or imprisonment, or both (18 U.S.C. 1001) and may jeopardize the validity of the application or any patent issuing thereon.

Dr. Keril J. Blight

August___, 2004

Electroparation Hun 7 B p60

WT/Ava. ING AS 10 MG pol / Nica. 1 Propt - Original (HCV/epBarMon: Ava II) ceilinar PNA (4-1-00) - logique vior and soly

- +10 TITES split 1:2 24thr prior to electroporation
- * Procedure as always (Plater) is & 3/3 on 2150's. Also, removed 3.5ml of fir
- * 4.2x 10 Tells total Resuspend 3ml D-185 => 15.5x10° cells/ell
- * 26hr post-electroporation and GAIE at Implimit
- * Try asing a pso dishes & seed on 8 well chamber slides for IF (no G-418
- . Diated remainder of ceils * Acetone fix + 8hr post-seeding SIF FOR NESS (1H7) => VEGATIVE! on a pico. Added GAIR - 16hr after sceding
 - -> The deletion is adaptive

Electroporation HUNT (CMR) p49

.-- Wr / Avo. 1 PUC: CV + 91197 HUM7B - prof / Avail - Criginal (-cures FortMan / Avall) certifican RNA(4-1-00) - roug certain RNA any

- * 8 TITES Split 1,2 25hir prior to electroporchan
- + Procedure as previously (Platect 1) + 73 on plans Also, removed a smit from
- * 9-2 x107 cells local > Resuspend 6ml 785 > 6x16 cells /EP
- * Albert post-electroporation add GA18 at U-Rmalmi
 - > Deletion is adaptive in CMR Hint's. In fact, more colonies are consistently observed for Havep BartMan & Havrep/Avo. in CMR Hunt cells is Borterischlager's.

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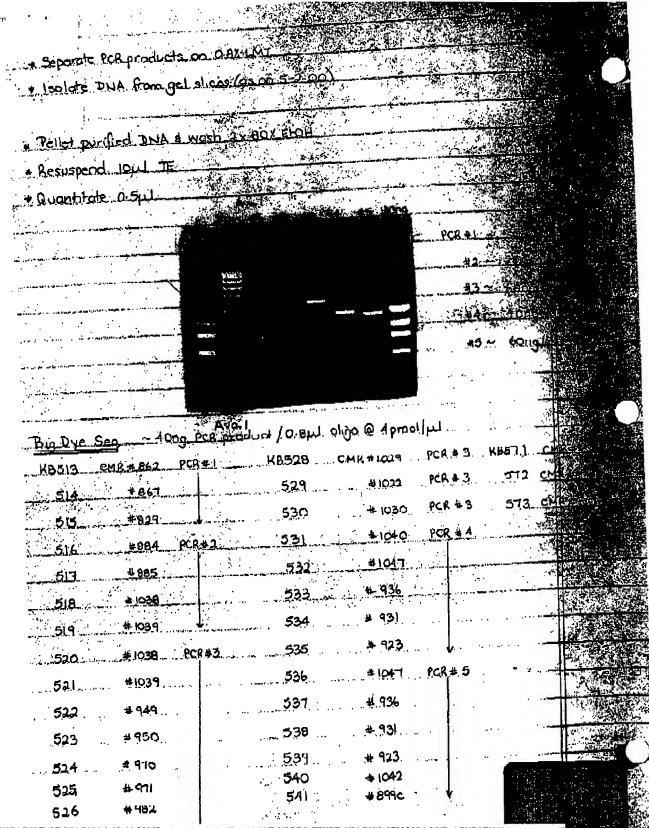
PAGE 13/37 * RCVD AT 8/30/2004 6:12:40 PM [Eastern Daylight Time] * SVR:USPTO-EFXRF-1/5 * DNIS:8729306 * CSID: * DURATION (mm-ss):11-12

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  CREATING NEW contig 1: from BartMan WT(1>8012)
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    ENTERING 25-KB510(18>703) in contig 1: percent match 29
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Page 1

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Big Dye Seg. ~40ng PCR product Ava. 5

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  ENTERING: 13.KB592(8>738) in contig 1: percent match 95
  ENTERING 14-RB593(1>576) in contig 1: percent match 94
ENTERING 15-RB594(1>708) in contig 1: percent match 95
  WITERING 16-KB595 (9>698) in contig 1: percent match 94
              17. KR596 (3>558) in contig 1: percent match 94
     PRING 18-KB597 (34>551) in contig 1: percent match 98
  ENTERING 19 KB598(20>545) in contig 1: percent match 98
  ENTERING 20-KB599(5>325) in contig 1: percent match 94
ENTERING 21-KB600(1>596) in contig 1: percent match 97
       NOT ENTERING in contig 2: 22. KB601(1>701) due to percent match (51) below threshold 65
  ENTERING 22-KB601(1>701) in contig 1: percent match 96
       NOT ENTERING in contig 2: 23-KB602(1>540) the to percent match (50) below threshold 65
   ENTERING 23 KB602(1>540) in contig 1: percent match 98
       NOT ENTERING in contig 2: 24-xB603(6>648) due to percent match (45) below threshold 65
   ENTERLING 24-KB603(6>648) in contig 1: percent match 98
   ENTERING 25-KB604(13>733) in contig 1: percent match 95
       NOT ENTERING in contig 2: 26 KB605(1>735) due to percent match (51) below threshold 65
   ENTERING 26 KB605(1>735) in contig 1: percent match 98

NOT ENTERING in contig 2: 27 KB606(1>603) due to percent match (50) below threshold 65

ENTERING 27 KB606(1>603) in contig 1: percent match 99
       NOT ENTERING in contig 2: 28 KB607(1>703) thue to percent match (45) below threshold 65
   ENTERING 28 KB607(1>703) in contig 1: percent match 95
ENTERING 29 KB608(17>727) in contig 1: percent match 96
ENTERING 30 KB609(14>11/) in contig 1: percent match 90
   EMPERING 31.KBG10(43>698) in contig 1: percent match 97
   Elapsed Time 0:0:17
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	Page
Tuesday, Project: Ava.5 slign Contig 1	10 5020
	ANALTINITICTACGGCCCTGTGGCGGGTGG-CT-GCTGAGGAGTTACGTGGAGGTTAC-GCGGGTGGXXXATTICCTACGAGGAGGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGA
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17.KB596(3>558) 19.KB598(20>545)	<- CCACTGACARCOTRANGUESCCOCCUSTACULUS
BartMan WI copy 1(1>6012) 18:EB597(34>551)	TGPANACCCTIOTINGAGARGARGATICACHTICOTROTROGOCTCANTONATACCTROTTINGGETCACAGCTCCATROCAGCCCGAACCGC ->TGCAAACCCCTCCTIACGGGAGGAGGTCACATTCCTROTROGGGCTCAATCAATACCTGGTTGGGTCACACTCCCATROCGAGCCGGAACCGC -> TGCAAACCCCTCCTTACGGGAGGAGGTCACATTCCTROTROGGCTCAATCAATACCTGGTTGGGTCACACTCCAATGCGAGCCCAATCCAATCAAT
19•KB598 (20>545)	<- IVX.094.00.00
BartMan WT copy 1 (1>8012) 17 • KB596 (3>558)	.1
20•X8599 (5>325)	5340 5350 5360 5370 5380 5390 5400 5410 5420 5340 5350 5360 5370 5380 5380 5390 5400 5400 5420 5340 5350 5360 5370 5380 5380 5380 5380 5380 5380 5380 538
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20•кв599 (5>325)	5430 5430 5450 5450 5470 5480 5430 5503 5513 5513 5530 5513 5530 5513 5530 5530
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Big Die Seg	~ 40ng IPCR product	Ava. 2
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		PCR #3	Bong/µl			
		PCR #4	40ng/pl			
:		PCR # 5 .	Lylenor			
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1	612	* 867		K8632	4 1041	
. į	613	#829	\downarrow	KB 633	± 936	
٠.		# 984	PCR#2	k863 4	*931	
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ਾ *	616	-8601 *		KB 635	# 1047	PCR \$5
4	617	#1039		637	# 936	PCR#5
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**	619	#1038	PCR#5.	639	# 9,23	
		P601#		620	41042	
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	622	± 170 # 170				
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BartMan WT copy(1>8012) 01-KB611(36>680) 03-KB613(11>666)	02•KB612(35>446) Barthan WI copy(1>8012) 01•KB611(36>680) 03•KB613(11>666)	02 • KB612 (35>446) BartMan Wr copy (1>8012) 01 • KB611 (36>680) 03 • KB613 (11>666)	02-KB612 (35>446) Barthan NT copy (1>8012) 01-KB611 (36>680) 03-KB613 (11>666)	02-KB612 (35>446) BartMan WT copy(1>8012) 01-KB611 (36>680) 03-KB613 (11>656)	(210	Wedneeday, Project: Untitled Contig 1
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BartMan WI copy(1>8012) 19-KB639(11>702) 20-KB630(23>335) 21-KB631(2>679) BartMan WI copy(1>8012) 21-KB631(2>679)	17.KB627(8>568) 20.KB630(23>335) BartMan W copy(1>8012) 19.KB629(11>702) 17.KB627(8>568) 20.KB630(23>335) 21.KB631(2>679)	178012)	Wr copy (1>8012) - (13>696) (11>702) (8>568)	Wednesday.
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Page

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Wednesday.
Untitled
Construction parameters:
   tch Size
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    rimm Added Cap Length in Contig
   kimum Added Gap Length in Sequence
                                                                 70
                                                                  65
Minimum Match Percentage
Maximum Register Shift Difference
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Lastgroup Considered
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Gap Penalty
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 Cap Length Penalty
                                                                  75
 Consensus Threshold
 CREATING NEW contig 1: from Bartman WT copy(1>8012)
 ENTERING 01.KB611(36>680) in contig 1: percent match 95
 ENTERING 02-KB612 (35>446) in contig 1: percent match 99
 ENTERING US-KBOIS(17>bob) in contig 1: percent match yy

Sequence 04-KB614 was not added, it is all poor data —

Sequence 05-KB615 was not added, it is all poor data —

Sequence 06-KB616 was not added, it is all poor data —

Sequence 06-KB616 was not added, it is all poor data —

Sequence 06-KB616 was not added, it is all poor data —

NOT ENTERING in contig 1: 07-KB61/(1>597) due to percent match (47) below threshold 65

NOT ENTERING in contig 1: 07-KB617(1>597) due to percent match (51) below threshold 65
 ENTERING 03-KB613(11>666) in contig 1: percent match 99
 CREATING NEW conting 2: from 07-88617(1>597)
 Sequence 08 KR618 was not added, it is all poor data
        NOT ENTERING in contig 2: 09 KB619(16>662) due to percent match (45) below threshold 65
 ENTERING 09-KB619(16>662) in contig 1: percent match 92
 ENTERING 10.KH620(9>517) in contig 1: percent match 96
ENTERING 11.KH621(65>627) in contig 1: percent match 96
Sequence 12.KH622 was not added, it is all poor data
  ENTERING 13-KB623(38>575) in contig 1: percent match 97
  ENTERING 14-xB624(6>414) in contig 1: percent match 92
  PNTERING 15-KB625(1>700) in contig 1: percent match 98
TERING 16-KB626(20>542) in contig 1: percent match 95
  TERING 17-KB627(8>568) in contig 1: percent match 94
ENTERING 18-KB628(38>696) in contig 1: percent match 96
  ENVISRING 19-KB629(11>702) in contig 1: percent match 96
  EXTERING 20.KB630(23>335) in contig 1: percent match 95
  EMTERING 21.KB631(2>679) in contig 1: percent match 98
NOT ENTERING in contig 2: 22.KB632(1>707) due to percent match (50) below threshold 65
  ENTERING 22. KB632(1>707) in contig 1: percent match 97

NOT ENTERING in contig 2: 23. KB633(1>721) due to percent match (51) below threshold 65

ENTERING 23. KB633(1>721) in contig 1: percent match 97
        NOT ENTERING in contig 2: 24 KB634 (7>642) due to percent match (47) below threshold 65
  ENTERING 24-RB634(7>642) in contig 1: percent match 98
ENTERING 25-KB635(13>726) in contig 1: percent match 97
         NOT EMTERING in contig 2: 26. KB536(1>680) due to percent match (50) below threshold 65
   ENTERING 26*KB636(1>680) in contig 1: percent match 98
NOT ENTERING in contig 2: 27*KB637(1>688) due to percent match (50) below threshold 65
  EMTERING 27-KB637(1>688) in contig 1: percent match 97
NOT EMTERING in contig 2: 28*KB638(12>658) due to percent match (45) below threshold 65
EMTERING 28*KB638(12>658) in contig 1: percent match 98
EMTERING 29*KB639(15>728) in contig 1: percent match 97
EMTERING 29*KB639(15>728) in contig 1: percent match 97
   EMPERING 30-KR640(8>135) in contig 1: percent match 85
EMPERING 31-KB641(1>666) in contig 1: percent match 86
   Elapsed Time 0:0:16
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Wednexday;

DNA StriderTM 1.3f7 ###

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TI PINE BI n.1. 5336

I Ant 5345-5485

I (Ava. 2) n.+.5320

Ava. 5 n.t. 3550 4 nt. 4573

n.t. 5290

na sequence 11313 by goodgesscooth .? egactosciata circular extensionlages repliced I377/NS3-NOTE (Genbenk AJ242652). Constructed in the pure backbone.

HCVrep1b BartMan/AvaII [1801 to 7758] -> Translate · 1-frame

Marked by AVETI in the variable region of the 3'NTR.

_= NS3 1831/11 age etc aca ggc cgg gat agg aac cag gtc gag gng gag gtc caa gtg dtc tce acc gca EOSDER O A E C E A O A A U P

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Ava. II/Clonec/Clone D n.t. 5336

n.t. 5320 Aug. 13) n.t. 5513 Ava.7 Huh. 2 n.t. 5314

CloneA CO9 -->- \$9º Arg Gln

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PAGE 30/37 * RCVD AT 8/30/2004 6:12:40 PM [Eastern Daylight Time] * SVR:USPTO-EFXRF-1/5 * DNIS:8729306 * CSID: * DURATION (mm-ss):11-12
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19-3-00

G418-colonies picked for BartMan/AvaI in CMR Hiti7 cells

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tuh.4 10µg \( \frac{1}{3} \) 8-2-00 \\
tuh.5 \quad \qu
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⇒ G418 added to 750 p.g/ml on 20-3-00

Huh, 4

Transfer to 24 well plate 21-3-00 pl

- Transfer to 6 well plate 22-3-00 pz

- Transfer to T25 flask 25-3-00 p3

ALSO, Cell count #1 3.2x105 cells/m1 32105 cells/m1

Cell count #2 8.95 x105 " 3x105 cells/m1

>> Trivol extract RNA from 80ml cells (2.4 x104 cells)

= Transfer to T75 flask 30-3-00 - 3p4

10-4-00 - Fraze 12 yrals @ 4x106 cells/vial Huh-4 pt (split 1: 28-4-00)

Tank 2 Rack 5 Box 1 (I vial); Box 6 (I vial); Box 7 (I vial);

Box 8 (6 vials); Box 9 (I vial) & Tank 2 Rock 7 Box 3 (2 vials)

Huh. 5

- Transfer to 24 well plate 24-3-00 Pl

- Transfer to 12 well plate 28-3-00 p2

-Transfer to 6 well plate 30-3-00 p3

-Transfer to T25 Flask 2-4-00 P4

ALSO, Cell count #1 4.4 x105 cells/ml 43x105



⇒ Trizal extract RNA from 80µ1 (~3.4×104 cells)

-Transfer to T75 flask -4-00 p5

20.4-00 - Froze 10vials 4×10 cells /vial p8 (3 T1756 split 1:15 on 1:1-4-01)

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Hun. t
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-Transfer to 24 well plate 22-3-00 pl
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- Transfer to 12 well plate 25-3-00. p2

- Transfer to 6 well plate 26-3-00 p3

- Transfer to T25 flask 29-3-00 p4

ALSO, Cell count #1 2.75 x 105 cells [m] } 3 x 105 culls/ml

> Trizal extract RNA from 80ml (2.4x104cells)

- Transfer to T75 flask, 2-4-00 p5

12-4:00 - Froze 12 vials @ 4x106 cells/vial Holiz 7 p8 (split 1:2 on 10-4-00)

Tank 2 Rack 7 Box3 (2 vials)

" Box 4 (+ vials)

Box5 (5 vials)

H.h. 8

- Transfer to 24 well plate 21-3-00 pl

- Transfer to 12 well plate 24-3-00 p2

- Transfer to 6 well plate, 26-3-00 p3

- Transfer to T25 flask 29-3-90 p4

ALSO, Cell count #1 14x105 cells/ml } 1.75x105 cells/ml

> Trital extract ANA from 85µ1 (~1.5×104 cells)

- Transfer in T75 flask 3-4-00 P5

14-4-00 Froze lovials @ 4x106 cells/vial Hub. 8 ip8 (split 1:2 12-4-00)

Tank 1 Rack 3 Box 2 (1 vial)

Box4 (I vial)

Box5 (Ivial)

Box6 (Ivial)

Tank | Rack 3 Box 4 (I vial)

Box 8 (Ivial)

Remainder stored in - 80°C Geezer bux



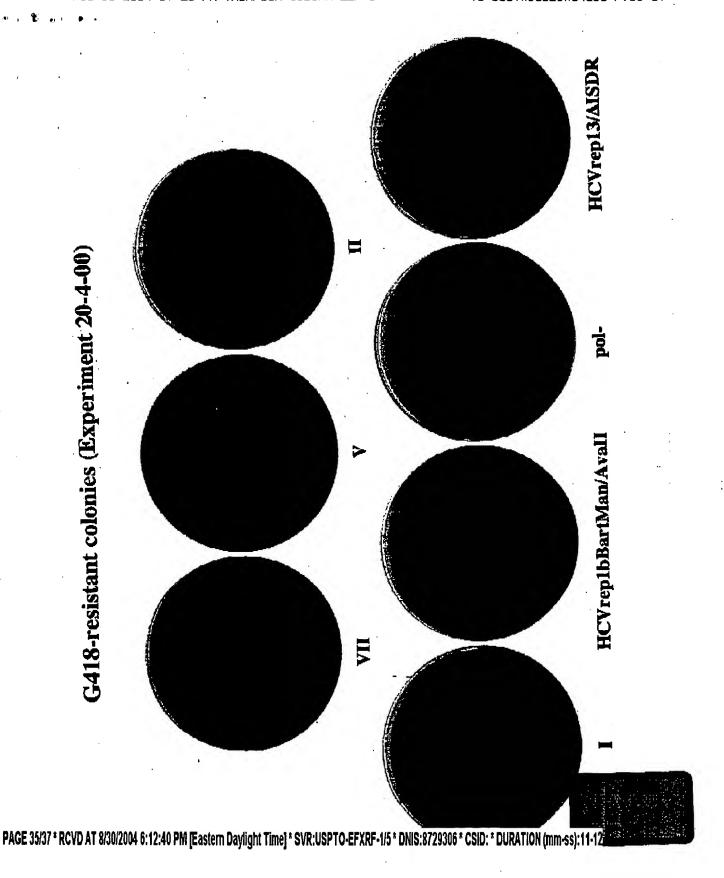
Transfer to 214 well plate 28-3-00 pl. - Transfer to 12 well plate 6-4-00 p2 mode Transfer to 725 flask 19-4-00 p3 - Transfer to 725 flask 19-4-00 p4 ALSO, Cell count = 1 6-9×10 cells/ml. 2 v8-5×105 Cell count = 2 106 cells/ml. 2 v8-5×105 Cell count = 2 106 cells/ml. 2 v8-5×105 Cell count = 2 106 cells/ml. 2 v8-100 p5 10-500 = Transfer to 175 flask 23-4-00 p5 Stored = 80:C flast cert box Clone D - Transfer to 24 well plate p2 - Transfer to 6 well plate p3 - Transfer to 6 well plate p3 - Transfer to 125 flask 16-3-00 p4 ALSO, Cell count = 1 3-4×105 cells/ml. 2 3×105 ce Cell caunt = 2 25×105 - Transfer to 175 flask 20-3-00 p5 31-9-00 = Trans 7 vials @ 3-5×106 cells/vialp8 (AT115) Tant 2 Rock 7 80×7	
Transfer to 12 well plate 6.4.00 p2 (mode) Transfer to 6 well plate 14.1.00 p3. Transfer to 725 flask 19.4.00 p4. ALSO, Cell count 2 6.9×10 cells/ml 2 85×105 Cell count 2 106 cells/ml 2 85×105 Transfer to 775 flask 23.4.00 p5. Incomposition of 4×106 cells/vial p8. Stored 80.0 flaster box Clone D - Transfer to 24 well plate p1. - Transfer to 6 well plate p3. - Transfer to 6 well plate p3. - Transfer to 725 flask 14.3.00 p4. ALSO, Cell count 2 3.4×105 cells/ml 23×105 cells cells m1. 23×105 cells m1. 2	المعقف دواعده دار
Transfer to 6 well plate 14-1-00 p3 Transfer to T25 flask 19-4-00 p4 ALSO, Cell count = 1 6-9×10 cells/ml Transfer to T15 flask 33-4-00 p5 Transfer to T15 flask 33-4-00 p5 LOSO - Transfer to 24 well plate p1 - Transfer to 12 well plate p2 - Transfer to 12 well plate p2 - Transfer to 6 well plate p3 - Transfer to 6 well plate p3 ALSO, Cell count = 1 3-4×10 cells/ml f3×10 cell caunt = 2 25×10 cells/ml f3×10 cells/ml f3×10 cell caunt = 2 25×10 cells/ml f3×10 cells/ml f3×1	change (12-4-00)
Transfer to T25 flask 19-4-00 pt ALSO, Cell count # 2 106 cells/ml Trizol extract RNA from 70 \(\text{-6x.10}^4 \text{ cells} \) Trizol extract RNA from 70 \(\text{-6x.10}^4 \text{ cells} \) Transfer to T15 flask 33-4-00 p5 10-5-00 - Froze 12. vials at 4x. 106 cells/vial p8 Stored -80.0 faster box Clone D Transfer to 24 well plate p2 - Transfer to 6 well plate p3 - Transfer to 6 well plate p3 - Transfer to T25 flask 14-3-00 p4 ALSO, Cell count # 1 3.4x. 105 cells \(\text{-3x.10}^5 \) \text{-cell caunt # 2 25 x 105} \text{- Transfer to T75 flask 20-3-00 p5} 31-3-00 - Froze 7 vials @ 3.5x. 106 cells \(\text{-7x.10} \) Transfer to T5 flask 20-3-00 p5	
ALSO, Cell count #1 6.9x10 cells/ml 285x105 Cell count #2 106 cells/ml 285x105 Trizal extract RNA from 70 µl (-6x104 cells) Transfer to T75 flask 23-4-00 p5 Stored -80.00 feeter box Clone D Transfer to 24 well plate p1 Transfer to 6 well plate p3 Transfer to 6 well plate p3 Transfer to T25 flask 16-3-00 p4 ALSO, Cell count #1 3.4x105 cells/ml f3x105 cell caunt #2 2.5x105 Transfer to T75 flask 20-3-00 p5 31-3-00 - Frace 7 vials @ 3.5x106 cells/vialp8 (AT175)	y - e - 1
Cell count # 2 106 cells/ml] Trizol extract RNA from 70 \(\tau \) \(\tau	ensimi
Trizol extract RNA from Topul (~6x10° cells) - Transfer to TT5 flask 33-4-00 p5 10-5-00 - Fraze 12 vials at 2x10° cells/vial p8 Stored - 80°C freezer box Clone D - Transfer to 24 well plate p1 - Transfer to 12 well plate p2 - Transfer to 6 well plate p3 - Transfer to T25 flask 16-3-00 p4 ALSO, Cell count \$1 3.4x10° cells/m1 23x10° cell caunt \$2.25 x10° Trizol extract \$0µL (2.7x10° cells) - Transfer to T75 flask 20-3-00 p5 31-3-00 - Fraze 7 vials @ 3.5x10° cells/vial p8 (47775)	and the same of the same of the same
-Transfer to T15 flask 33-4-00 p5 10-5-00 - Fraze 12-vials at 4x106 cells/vial p8 Stored -80°C flaeter box Clone D -Transfer to 24 well plate p! -Transfer to 6 well plate p2 -Transfer to 6 well plate p3 -Transfer to T25 flask 16-3-00 p4 ALSO, Cell count #1 3.4x105 cells m! / 3x105 cell caunt #2 2.5 x105 >Trizal extract 80pt (2.7x104 cells) -Transfer to T75 flask 20-3-00 p5 31-3-00 - Fraze 7 vials @ 3.5x106 cells/vial p8 (4.7175)	<u>) ,</u>
Clone D Clone D Transfer to 24 well plate pl Transfer to 6 well plate p3 Transfer to 125 flask 163-00 p4 ALSO, Cell count #1 3.4x105 cells m1 / 3x105 cell caunt #2 2.5x105 Transfer to 775 flask 20-3-00 p5 31-8-00 - Transfer to 7 vials @ 3.5x106 cells /vial p8 (ATITE	g.
Stored - 80°C feeter box Clone D - Transfer to 24 well plate pl - Transfer to 12 well plate p2 - Transfer to 6 well plate p3 - Transfer to 725 flask 16-3-00 p4 ALSO, Cell count *1 3-4×10 ⁵ cells m1 / 3×10 ⁵ cell caunt #2 2-5×10 ⁵ > Trizal extract 80µL (2-7×10 ⁴ cells) - Transfer to 775 flask 20-3-00 p5 31-3-00 - Trace 7 vials @ 3-6×10 ⁶ cells /vial p8 (4-7) =	I consequently and the second
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- Transfer to 24 well plate p2 - Transfer to 6 well plate p3 - Transfer to 725 flask 16-3-00 p4 ALSO, Cell count #1 3.4×10 ⁵ cells m1 / 3×10	
- Transfer to 12 well plate p3 - Transfer to 6 well plate p3 - Transfer to 725 flask 16-3-00 p4 ALSO, Cell count #1 3.4×10 ⁵ cells m1 / 3×10	
- Transfer to 6 well plate p3 - Transfer to 725 flask 16-3-00 p4 ALSO, Cell count #1 3.4×10 ⁵ cells m1/3×10 ⁵ cells	
- Transfer to 725 flask 16-3-00 p4 ALSO, Cell count #1 3.4×105 cells m1 / 3×105 cell Cell count #2 2.5×105 Trizel extract 80µl (2.7×104 cells) - Transfer to 775 flask 20-3-00 p5 31-9-00 - Trace 7 vials @ 3.5×106 cells/vial p8 (4.7175)	
ALSO, Call count #1 3.4x105 cells m1 } 3x105 ce Call count #2 2.5 x105 Trizal extract 80µ1 (2.7x104 cells) -Transfer to T75 flask 20-3-00 p5 31-3-00 - Trace 7 vials @ 3.5x106 cells/vial p8 (4.7175)	
Cell count #2 2.5 x 105 → Trizal extract 80µl (2.7x 104 cells) -Transfer to T75 flask 20-3-00 p5 31-3-00 - Trans 7 vials @ 3.5x 106 cells/vial p8 (4.7175)	
> Trizal extract 80µl (2.7x104cells) - Transfer to 775 flask 20-3-00 p5 31-5-00 - Trace 7 vials @ 3.5x106 cells/vial p8 (4.7175	iralum.
- Transfer to T75 flask 20-3-00 p5 31-3-00 - Trace 7 vials @ 3-5×106 cells/vialp8 (47175	
31-9-00 - Frace 7 vials @ 3-5×106 cells/vial p8 (4-17)=	
	's split 1:2 on 2
Lank & nake 1 and a second control of the se	
\mathbb{N}	
	mineral energy of any time and apply the mean reason
19 7	San

20-4-00

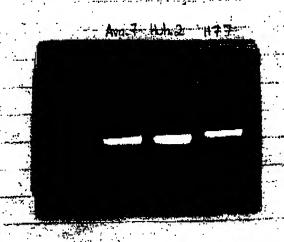
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Electroporation Hubit (CMR) p64

—	The second secon
	- bx 10 ce 11s/electroporation
	t 1.5µg replicar ANA + 5µg Hoh78 cellular ANA (4-1-00)
 	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
	(1) HCVrep/Ava.2 BB V
	(2) HCVrep/Ava. 5 BBI
	(3) HCVrep/Clone B BB VII
	4) HCV rep / Ayo.! BB.T
	(5) HCKmp18/ AISDR
, a manage of 5 ments of 5	(6) House Bort Man / Ava I (anginal).
	(F) HCVIEP(pol-)/Avail
	(8) Poliorep-GFP
	and the second s
	Place electroporated cells into media. Total volume = 9.5ml
***	A. Plate Q-5m1/p.100.
	B. Plate 3ml & 6ml per p150
ppose track press track	
21-4-9	Denomination of the second of
***************************************	At G418 at 0.8 mg/ml - 27hr post-electroporation
, ,	
	Stain p100's on 6-5-00
ma saa	Stam place with 3ml placed on 14-5-00
	N.B. When the cells are plated too dense they begin to detach
	due to overconfluence, particularly Clone B & Ava. 2 which
	appear to replicate in >90% of thin 7 cells
	Clone 8 > Ava. 2 > Ava. 5 & Ava. 1 + of Gal 18-resistant colonies
;	No colonies observed for 477 HCV(cep13/AISDR & HCV/cep(polt)/Ava. 1
* RCVD AT 8/30/2004 6:	12:40 PM [Eastern Daylight Time] * SVR:USPTO-EFXRF-1/5 * DNIS:8729306 * CSID: * DURATION (mm-ss):11-12



check the (- dug) on non-denoturing get



21-5-00

Electroporation Hin7 p63

5 Tins split 1/2 ~ 24hr prior...........

1-549 Hourepana + 449 Hubits cellular ANA (4-1-00)

~ 6 x 106 cells/electroporation.

- I. Hevrepib/Ava. 1 BBI
- .a. Hevreplb/Ava. 2 BB Y
- 3. Heureph Ava. 5 BBI
- 4 Hovreph Ava. 7 BBIY
- 5 Hovreplb/Clone B BBVT .
- 6. HCyrep 16/Huh. 2. BBIII
- 7. HCVrep13/Ser→Ile(1179)
- & HCV, rep. 16 BortMan/AvaI (original)
- 9. Hovreply Bartman (pol-)/AvaII...



....Valume total or 9.5 ml

= Plate 0.4ml, 0.0ml, 0.2ml, 0.1ml per ploa.

Plate 15ml per p150

(N.B. Also plained 6ml/piso for Herropis/ser +The)

PAGE 36/37 * RCVD AT 8/30/2004 6:12:40 PM [Eastern Daylight Time] * SVR:USPTO-EFXRF-1/5 * DNIS:8729306 * CSID: * DURATION (mm-ss):11-12

22-5-00	
Electroparation Hela cells p15	
	4
5 7175's split 30hr prior 1:2	
1 13 VIO cells total resuspended in 8.5ml Isc cold Depos	
k 99 pises, 0.9 kV, 5 pulses, 0.4 ml cells (~ bx 106 cells)	
2 mg HCVrcp RNA	
	g. 444 987 9
1 Herreple/Clane B BB VII	<u> </u>
o HCV-plb/Ava.1 BB7	
3 Hevrepib Ava. 2 Bay	
4 Hevrepth /Ava.5 BBT	<u></u> . :
HCV rep16 /Ava. 7 BBTU	
6 11CV rep 16/Huh 2 BBIII	
# HCVcep1bBartMan/AvaT	
8 HCV-coll BortMan (pa)-)/AvaI	·
9 HCM rep 13/5 >I	
ia Hevrep 13/AISDR	
No RNA	
disc.	
* V_ ~ 9.5ml media + EPed cells.	
	ļ <u>. </u>
* Plate 3ml & 6ml per place (For no RMA EP, plated total cells on	p150)
4-5-00 A+ 48hr post-EP add 0.8 mg/ml G418	
At Ann post-EP add U.s alland	
C. C. Ib. Quian electroporated	
> No colonies absenced for any of the RNAs cleatopporated	
	and the state of t

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